acetone bodies in fasted rats upon feeding acetic acid.[4] By the use of heavy carbon labeling of acetic acid final proof of its conversion into the acetone bodies was established by Swendseid *et al.*[9] Although acetic acid is ketogenic in the fasting animal it has been found by MacKay (personal communication) and also in this laboratory that it will not form acetone bodies in the fed animal. MacKay, Wick and Barnum[10,11] in studying acetone body formation from the odd carbon-fatty acids, and short chain even numbered carbon fatty acids have found that if the chain contains 4 to 10 carbons, acetone bodies are formed even in the presence of normal liver glycogen. This gives ample proof that ketogenesis can, under certain conditions, go on in the fed animal. Furthermore, the fact that valeric acid forms acetone bodies under such conditions gives additional evidence that the acetone body formation takes place through a 2-carbon compound condensation.

There are 4 possible 2-carbon acids that may be formed by β-oxidation of a fatty acid. These are acetic, glycolic, glyoxylic, and oxalic acids. In order to fulfil the qualifications of the modified β-oxidation hypothesis, the 2-carbon acid must be capable of acetone body formation in the fed animal. Experiments which have been performed in this laboratory have shown that oxalic acid is too toxic for a study of acetone body formation. Neither acetic nor glycolic acids will form acetone bodies in the fed animal which leaves glyoxylic as the only possible intermediate of this type. It is possible that a preliminary reduction or some other reaction of the fatty acid prior to its cleavage might take place, and in such an event other intermediates might exist. However, as a working hypothesis it is proposed that in the catabolism of fatty acids glyoxylic is formed and under the proper conditions this compound is converted into the acetone bodies by the liver.

Summary. It has been shown that in the fasted rat glycolic acid does not form glycogen nor does it form acetone bodies in the fed or fasted rat. Glyoxylic acid causes the formation of acetone bodies in the fed rat. From this evidence it has been concluded that neither glycolic nor glyoxylic acids are intermediates in the transformation of glycine to glucose in the animal body.

9 Swendseid, M. E., Barnes, R. H., Hemingway, A., and Nier, A. O., *J. Biol. Chem.*, 1942, **142**, 47.

10 MacKay, E. M., Wick, A. N., and Barnum, C. P., *J. Biol. Chem.*, 1940, **135**, 183.

11 MacKay, E. M., Wick, A. N., and Barnum, C. P., *J. Biol. Chem.*, 1940, **136**, 503.

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Correlation of Extent of Tuberculosis with Amount of Polysaccharide in the Serum.*

FLORENCE B. SEIBERT, J. WALTER NELSON AND MABEL V. SEIBERT.
From the Henry Phipps Institute, University of Pennsylvania, Philadelphia.

An intensive study has been made in recent years of tuberculous sera, with the objective of detecting the presence of antigen, antibody or any substance which may be characteristic of the disease. The primary approach has been based upon observation of the electrophoretic diagrams of sera by means of the Tiselius technic.

It was shown[1] that during the development of tuberculosis in the rabbit the beta globulin fraction increased in proportion to the extent of the disease. In the human being, on the other hand, the consistent change[1] was a progressive increase in the gamma globulin. Increases in alpha and beta globulin fractions

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1 Seibert, F. B., and Nelson, J. W., *Am. Rev. Tuberc.*, 1942, **47**, 66.

TABLE I.
Amount of Polysaccharide in Fractions of Serum.

Sera fractionated	% of polysaccharide in the Albumin	Gamma globulin	Whole serum (dialyzed)
A.H.—Far advanced tuberculosis	3.3	2.4	2.4
P.R.—Pleural fluid, far advanced tuberculosis	3.1	2.4	
M.M.—Extensive sarcoidosis	2.2	1.2	
No. 78—Normal rabbit serum	1.0	1.0	

also frequently occurred but not with the same degree of regularity as was the case with the gamma globulin.

An explanation of the cause of the rise in any of these components would be of value in the understanding of the disease. The possibility of the presence of antibodies immediately presents itself in connection with the rise in the gamma globulin, especially in view of the fact that antibodies to the tuberculin protein have been definitely demonstrated[2] to be present in this fraction in sensitized rabbits. However, there could not be shown to be sufficient antibody to account for the total rise in the gamma globulin.

In seeking for other explanations of the rise, one is impressed with the idea that if tuberculin polysaccharide were found free in the serum, it would migrate in electrophoresis with approximately the same mobility as the gamma globulin.[3] Analyses were, therefore, made of the polyssacharide content of the albumin and the gamma globulin fractions isolated from several sera by means of electrophoresis.

The polysaccharide was determined directly on the protein solutions, without preliminary hydrolysis, by means of the carbazole reaction,[4] as outlined previously,[5] using the Evelyn colorimeter. Table I shows the percentage of polysaccharide in the isolated albumin and gamma globulin fractions. These results are in the same range of values reported by other investigators. For example, Kekwick[6] found that the A fraction of human serum albumin contained 1.95% polysaccharide, and Hewitt[7] stated that the carbohydrate content of the total albumin fraction of serum was usually about 2.8%. Blix, Tiselius and Svensson[8] found 1.1 to 1.2% mannose in the albumin and 3.6 to 3.0% in the gamma globulin fraction of normal sera. In a pneumonia serum there was considerably more carbohydrate in the albumin (5.8%). Nilsson[9] reported a rise in the amount of carbohydrate bound to protein in the albumin as well as globulins of sera in pneumonia.

From the results in Table I it is clear that the amount of polysaccharide in both albumin and gamma globulin was higher in the serum (A.H.) and the pleural fluid (P.R.) both from cases of far advanced tuberculosis, than in the serum (M.M.) which was from a severe case of sarcoidosis, or than in the serum (No. 78) of a normal rabbit. It also becomes clear from these results that the obvious rise usually found in the gamma globulin fraction of sera of tuberculous patients is not due primarily to the presence of tubercle bacillus polysaccharide. Instead the polysaccharide which is present appears to be able to attach itself to all the serum protein fractions and possibly to the largest extent to the albumin. It does not easily separate from the proteins since these fractions have all been dialyzed before analysis. The serum (A.H.), when subjected undiluted to electrophoresis at pH 7.6 $\mu = 0.1$, showed almost immediately a definite separation of

2 Seibert, F. B., and Nelson, J. W., *J. Am. Chem. Soc.*, 1943, **65**, 272.

3 Seibert, F. B., and Watson, D. W., *J. Biol. Chem.*, 1941, **140**, 55.

4 Dische, Z., *Mikrochemie*, 1930, **8**, 4.

5 Seibert, F. B., *J. Biol. Chem.*, 1940, **133**, 593.

6 Kekwick, R. A., *Biochem. J.*, 1938, **32**, 552.

7 Hewitt, L. F., *Biochem. J.*, 1936, **30**, 2229.

8 Blix, G., Tiselius, A., and Svensson, H., *J. Biol. Chem.*, 1941, **137**, 485.

9 Nilsson, I., *Bioch. Zeits.*, 1937, **291**, 254.

TABLE II.
Correlation of Carbohydrate Content of Sera with Diagnoses.

Diagnoses	Symptoms	Total Carbohydrate, mg%	Glucose, mg%	Polysaccharide, mg%
F.S. Normal		194	97	97
M.S. "		194	82	112
J.N. "		181	51	130
H.T. "		158	70	88
I.W. "		157	57	101
C.C. "		185	84	101
J.R. "		195	75	120
W.L. "		200	80	120
F.V. "		209	82	127
W.S. "		187	89	98
R.M. "		216	92	124
T.W. "		204	78	126
E.R. "		211	78	133
C.E.B. Reinfection tuberculosis—Minimal	None	226	94	132
T.G. " " "	Moderate	205	76	129
C.T. " " " ; stationary	Slight	213	81	132
V.O. " " " ; bilateral	None	208	78	130
L.J. " " "	"	226	82	144
W.A. " " " "	"	227	71	156
P.D. " " " "	"	275	114	161
J.U. " " "	"	238	76	162
H.W. " " " "	"	230	58	172
J.H.Jr. " " " "	"	263	79	184
J.H. Reinfection tuberculosis—moderately advanced (rarefaction cleared)	"	231	115	116
E.W. Reinfection tuberculosis—moderately advanced, cavity closing (?), bilateral	Slight	229	85	144
A.N. Reinfection tuberculosis—moderately advanced	None	225	78	147
P.B. Reinfection tuberculosis—moderately advanced; progressive; bilateral	Hemoptysis	227	78	149
J.B. Reinfection tuberculosis—moderately advanced; bilateral	Slight	236	74	162
C.W. Reinfection tuberculosis—moderately advanced; progressive; bilateral	"	270	93	177
T.B. Reinfection tuberculosis—moderately advanced; cavity; bilateral	Moderate	232	71	161
L.V. Reinfection tuberculosis—far advanced; cavity closed later	Slight	181	74	107
H.P. Reinfection tuberculosis—far advanced since 1932	"	214	78	136
J.F. Reinfection tuberculosis—far advanced; bilateral	"	243	80	163
W.J. " " " " "	Moderate	237	64	173
R.B. " " " " "	"	284	90	194
A.H. " " " " " progressive; died in 3 mo.	Severe	293	106	187
J.N. Reinfection tuberculosis—far advanced; bilateral; progressive; died next day	Moderate	307	110	197
S.H. Reinfection tuberculosis—far advanced; bilateral; progressive; died in 9 days	"	334	123	211

Note—Statistical analyses show the differences of the polysaccharide results from the normal to be highly significant.

the albumin peak. Evidence of similar heterogeneity has been noted in many of the sera from patients with far advanced tuberculosis but not in normal sera. Blix, Tiselius and Svensson[8] mentioned a similar experience with pneumonia serum, and found that the faster fraction contained the larger percentage of carbohydrate.

It is of interest that the polysaccharide found in tubercle bacilli or tuberculin, which has been shown by Renfrew,[10] Dorset and

[10] Renfrew, A., *J. Biol. Chem.*, 1930, **89**, 619.

Henley,[11] Chargaff and Anderson,[12] and Heidelberger and Menzel[13] to consist of mannose, arabinose and galactose, gives a yellowish pink color[14] with the carbazole reagent, whereas glucose gives a bright pink color. The polysaccharide which can be detected in these serum protein fractions gives a similar yellowish pink color† and it has been shown by Rimington,[15] Bierry[16] and Hewitt[17] that the carbohydrate complex which they have found in serum proteins consists of mannose, galactose and glucosamine. Because of the similarity in composition in these two polysaccharides and in the color produced with the carbazole reagent, it was justifiable to use the standard tuberculin polysaccharide curve for translating the colorimeter readings into milligrams of polysaccharide. It is interesting to speculate whether the polysaccharide, found associated with the serum proteins and which increases during tuberculosis, is a normal constituent of the body, or whether it may be a foreign product, possibly produced by a pathogenic organism such as the tubercle bacillus. An answer might be reached by the application of a micro quantitative test for arabinose, which is a characteristic constituent of the tubercle bacillus polysaccharide.

Because of the suggestive nature of the results obtained it appeared worthwhile to determine in a simple way whether or not the polysaccharide content of whole serum increases in tuberculosis. Accordingly, total polysaccharide was determined on whole serum by applying the carbazole reaction[5] directly. Since the total carbohydrate of serum would include free glucose, the latter was determined on a second aliquot and the result subtracted from the total polysaccharide, giving the true polysaccharide content. In these studies the Shaffer-Hartmann reagent was used for determining the glucose, but any other approved method for glucose could be used instead. The purpose of these analyses was comparative rather than the determination of absolute values. Duplicate determinations could be run on a total of 2 ml of whole serum.

Analyses made in this manner on the sera of a series‡ of normal, minimal, moderately advanced, far-advanced and terminal tuberculous cases, show (Table II) that the true polysaccharide content of serum does increase during tuberculosis, and to some extent proportionate to the extent of the disease. The increase could not be correlated with an increase in total protein content of the serum.

Correlation of the polysaccharide values with the clinical course of the disease has in many cases been highly suggestive. For example, J. H. in the "moderately advanced" group showed considerable clearing of the lesion on X-ray and a low value for polysaccharide, whereas C. W. showed progression in the lesion and a high polysaccharide value. L. V. in the "far advanced" group also showed great improvement, with closure of the cavity, and the polysaccharide value was practically normal, whereas several others who showed progression of disease and soon died from tuberculosis had extremely high polysaccharide values. While the average polysaccharide value among the "minimal" cases is higher than among the "normals," the values vary from a high normal to those found among the "far advanced" and some of the latter give the impression of clinical danger. However, the suggestive prognostic value of the test can not be established until many such cases are followed over a sufficient period of time in order to determine the actual course of the disease.

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12 Chargaff, E., and Anderson, R. J., *Z. physiol. Chem.*, 1930, **191**, 172.

13 Heidelberger, M., and Menzel, D. O., *J. Biol. Chem.*, 1937, **118**, 79.

14 Seibert, F. B., Pedersen, K. O., and Tiselius, A., *J. Exp. Med.*, 1938, **68**, 413.

† Probably due to mannose which gives a similar color.

15 Rimington, C., *Biochem. J.*, 1931, **25**, 1062.

16 Bierry, H., *Compt. rend. soc. biol.*, 1928, **99**, 1837.

17 Hewitt, L. F., *Biochem. J.*, 1936, **30**, 2229.

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